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(54) Title: SPECIFIC BINDING MEMBERS AND USES THEREOF

(57) Abstract: The invention relates to the use of a binding member which binds to both SCR1 and SCR2 of CD55 in the treatment of tumours and leukaemia. The binding member may be an antibody which binds to SCR1 and SCR2 of CD55 and neutralising CD55 and making cancer cells susceptible to complement mediated attack.

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1     **Specific Binding Members and Uses Thereof"**

2

3     The present invention relates to specific binding  
4     members and their use in therapy. In particular, the  
5     invention relates to specific binding members which  
6     bind to CD55, their use in the modulation of  
7     complement activation and the treatment of disease,  
8     for example, neoplastic disease.

9

10    The human complement system consists of a highly  
11    efficient recognition and effector mechanism that  
12    consists of 30 serum or cellular components  
13    including activated proteins, receptors and positive  
14    and negative regulators. In brief, the complement  
15    cascade consists of a triggering step, an  
16    amplification step with a feedback loop and finally,  
17    a membrane attack or lytic step. The central  
18    component of the complement system is C3. Generation  
19    of C3b by the classical or alternative pathway is  
20    crucial for opsonisation and lysis. The classical  
21    pathway is initiated when component C1 via its Clq  
22    subcomponent attaches to an antibody to form an

1 immune complex. For the alternative pathway,  
2 however, there is no initiating factor equivalent to  
3 antibody. Rather it is in a state of continuous,  
4 low level activation as a result of spontaneous  
5 hydrolysis of a thioester group in native C3. This  
6 results in binding of C3 to non-specific acceptor  
7 molecules in plasma or on cell surfaces. This can  
8 result in the formation of C3 convertases and  
9 creation of a feedback loop. Because of its potent  
10 pro-inflammatory and destructive capabilities, there  
11 is a regulatory system designed to prevent  
12 complement activation both in the fluid phase and on  
13 bystander tissues.

14  
15 There are four membrane bound complement regulatory  
16 proteins namely complement receptor 1 (CR1), CD55,  
17 CD46 and CD59 (Liszewski et al 1996. Adv Immunol  
18 61:201-283). Regulation is either accomplished by:

- 19  
20 1. Spontaneous decay of activated proteins and  
21 enzyme complex (i.e. short half life)  
22 2. Destabilisation and inhibition of activation  
23 complexes  
24 3. Proteolytic cleavage of "activated" components.

25  
26 CD46, CD55 and CD59 are widely expressed on many  
27 tissues, including surface epithelia and tumour  
28 tissues. In contrast, CR1 expression is limited to  
29 peripheral blood cells and is therefore not directly  
30 involved in protection of solid tumours.

31

1 Most tumours are of epithelial origin and, although  
2 most surface epithelia express complement regulatory  
3 proteins, tumours show variable expression of CD55,  
4 CD46 and CD59. The majority of colorectal and  
5 thyroid cancers express high levels of all three  
6 complement regulatory proteins (Niehans et al., 1996  
7 Am J Pathol 149:129-142; Li et al., 2001 Br. J.  
8 Cancer 84:80-86; Thorsteinsson, 1998 APMIS 106:869-  
9 878; Yamakawa et al., 1994 Cancer 73:2808-2817).  
10 Ductal carcinoma of the breast shows the most  
11 variation in phenotype with some tumours expressing  
12 only one inhibitor while others express different  
13 combinations of two or three inhibitors (Niehans et  
14 al., 1996 supra; Thorsteinsson et al., 1998 supra).  
15 Renal cell carcinoma has weak to moderate expression  
16 of one to three inhibitors, generally CD55 and CD59  
17 (Niehans et al., 1996 supra) whereas non-small cell  
18 lung carcinomas and ovarian and cervical cancers  
19 usually express CD59 and CD46 with variable CD55  
20 immunoreactivity (Niehans et al., 1996 supra; Bjorge  
21 et al., 1977 Cancer Immunol Immunother 42:185-192;  
22 Simpson et al., 1997 Am J Pathol 151:1455-1467).  
23 Similar results have been obtained with established  
24 cell lines (Bjorge et al., 1996 supra; Gorter et al  
25 1986 Lab Invest 74 1; Juhl et al., 1997 J. Surgical  
26 Oncol. 64:222-230; Li et al., 2001 supra).  
27  
28 All three complement regulatory proteins are  
29 expressed on vascular endothelium. Their specific  
30 roles during inflammation when the risk of  
31 complement mediate injury may be increased remains  
32 to be determined. CD55, but not CD46 or CD59, is

1 up-regulated on endothelial cells by the pro-  
2 inflammatory mediators  $\text{TNF}\alpha$ ,  $\text{IL-1}\beta$ , and  $\text{IFN-}\gamma$ , and  
3 also by the MAC (membrane attack complex) and  
4 thrombin. These results suggest that CD55 is of  
5 critical importance in protecting endothelial cells  
6 from complement during inflammation and coagulation.  
7 Furthermore it has recently been shown that  
8 retraction of endothelial cells exposing sub-  
9 endothelial extracellular matrix is a potent inducer  
10 of the alternative complement pathway releasing  
11 anaphylatoxins that stimulate inflammation. As  
12 tumours frequently have disregulated endothelium,  
13 with exposed vessel walls, the tumour environment  
14 may induce complement activation. This may be one  
15 of the reasons that tumour cells over-express  
16 complement regulatory receptors. However, it has  
17 been shown that both tumour cells and endothelial  
18 cells can actually secrete CD55 but not CD46 into  
19 their extracellular matrix (ECM) (Hindmarsh and  
20 Marks, 1998 J. Immunol. 160:6128-6136). Hindmarsh  
21 and Marks showed that tumour but not endothelial  
22 derived CD55 is functionally active and can prevent  
23 deposition of C3b. However, deposition of matrix  
24 CD55 could not be up-regulated by inflammatory  
25 cytokines. More recently the present inventors have  
26 shown that both CD55 and CD59 can be deposited into  
27 extracellular matrix by both tumours and endothelial  
28 cells and the latter can be considerably up-  
29 regulated by the potent angiogenesis growth factor  
30 VEGF (Li et al., 2001 *supra*). Furthermore, CD55  
31 deposited by endothelial cells stimulated with VEGF  
32 was shown to be functionally active. VEGF is

1 unusual, as it is the only cytokine identified to  
2 date that up-regulates both cell surface expression  
3 and deposition of CD55 into the ECM.

4  
5 As most tumours secrete high levels of VEGF to  
6 induce angiogenesis they will stimulate expression  
7 of CD55 on endothelial cells and within ECM.  
8 Interestingly immunohistochemistry of colorectal  
9 tumours with anti-CD55 monoclonal antibodies shows  
10 intense staining of tumour stroma (Li *et al.*, 2001  
11 *supra*; Simpson *et al.*, 1997 *supra*; Niehans *et al.*,  
12 1996 *supra*) and blood vessels (Niehans *et al.*, 1996  
13 *supra*). CD55 deposited within ECM is covalently  
14 bound as it cannot be released by strong acids or  
15 alkalis.

16  
17 CD55 binds C3 convertases from both the classical  
18 and alternative complement pathways displacing C2b  
19 and C3b respectively. It can, therefore, prevent  
20 C3b deposition and inhibit the downstream assembly  
21 of the membrane attack complex. CD55 has an  
22 extracellular domain that is composed of 4  
23 contiguous short consensus (SCR) domains and a  
24 threonine/serine rich region proximal to the cell  
25 surface. It has a single N-glycosylation site  
26 between the first and second SCR domains and is  
27 heavily O-glycosylated in the threonine and serine  
28 rich regions. It is attached to the cell membrane  
29 by a glycosphosphoinositol (GPI) anchor and is  
30 expressed by all cells exposed to complement,  
31 namely, red blood cells, leukocytes, endothelial and  
32 epithelial cells. CD55 has also been detected in

1 low amounts in plasma, saliva and urine. The  
2 biological significance of this soluble form remains  
3 unclear as it has never been shown to be  
4 functionally active. Recently it has been shown  
5 that HeLa cells and HUVEC incorporate CD55 into  
6 their extracellular matrix and that this covalently  
7 linked CD55 can inhibit C3b deposition and the  
8 release of the pro-inflammatory anaphylatoxin C3a  
9 (Hindmarsh and Marks, 1998 *supra*).

10

11 As well as making tumour cells susceptible to *in*  
12 *situ* complement activation, antibodies inhibiting  
13 the functions of complement regulatory proteins may  
14 also make tumour cells susceptible to monoclonal  
15 antibody mediated complement dependent cellular  
16 cytotoxicity. A chimeric anti-LewisY monoclonal  
17 antibody (cH18A) mediated modest complement mediated  
18 cell lysis of two lung adenocarcinomas cell lines.  
19 However addition of antibodies that block the  
20 function of CD46, CD55 and CD59 considerably enhance  
21 complement mediated lysis. Use of multiple blocking  
22 antibodies to the complement regulatory proteins  
23 produced more enhancement of cH18A mediated lysis  
24 than any single antibody (Azuma *et al.*, 1995. *Scand*  
25 *J Immunol* 42:202-208). Several groups have generated  
26 bispecific antibodies with one arm targeting a  
27 tumour cell surface antigen and the other targeting  
28 the functional domain of a complement regulatory  
29 protein. A bispecific antibody targeting HLA and  
30 SCR3 of CD55 resulted in a 92% enhancement of C3b  
31 deposition on renal tumours. Similarly in the same  
32 study a bispecific antibody targeting a renal tumour

1 antigen and the SCR3 of CD55 resulted in a 25-400%  
2 increase in C3b deposition on renal tumours and  
3 rendered the cells susceptible to complement  
4 mediated lysis (Blok et al., 1998 J Immunol  
5 160:3437-3443). Finally when a chimeric anti-CD37  
6 monoclonal antibody was used to activate the  
7 classical complement pathway, a bispecific Fab'gamma  
8 construct targeting a lymphoma specific antigen and  
9 the CD59 functional domain increased cell lysis by  
10 3-5 fold (Harris et al., 1997 Clin. Exp. Immunol.  
11 107:364-371).

12

13 However, although previous studies have shown that  
14 monoclonal antibodies recognising SCR3 of CD55 could  
15 partially neutralise CD55 leading to enhanced C3b  
16 deposition and assembly of the MAC complex, each of  
17 these antibodies merely compete for binding to SCR3  
18 with the C3 convertases and therefore only partially  
19 neutralise CD55. Molecular constructs of CD55 have  
20 shown that SCR3 is the active domain of CD55 and  
21 that SCR2 and SCR4 are necessary to provide the  
22 correct conformation for C3 binding. No role for  
23 SCR1 in complement decay has been shown. However,  
24 although SCR2 is necessary to provide the correct  
25 conformation for C3 binding, studies with monoclonal  
26 antibodies to single SCR domains of CD55 have shown  
27 that only monoclonal antibodies that bind to SCR3  
28 and not antibodies that bind to either SCR1 or SCR2  
29 can neutralise CD55 (Coyne et al, 1992 J Immunol  
30 149, 2906).

31



1     Imaging studies with the monoclonal antibody 791T/36  
2     (Embleton et al 1981 Br.J. Cancer 43:582-587) in  
3     osteosarcomas, ovarian and colorectal tumours  
4     successfully imaged lesions as small as 1cm<sup>3</sup>  
5     (Farrands et al 1982 Lancet 2:397-400; Farrands et  
6     al 1983. J. of Bone and Joint Surg. 65:638-640;  
7     Armitage et al., 1985. Nucl Med Commun 6:623-631).  
8     Furthermore autoradiography of the resected tumours  
9     showed both cell surface and intense stromal  
10    localisation of the antibody (Armitage et al., 1984  
11    Br J Surg 71:407-412). These studies illustrate that  
12    an anti-CD55 antibody can effectively localise in  
13    tumours without showing any normal tissue toxicity.  
14    In particular no detectable binding of radiolabeled  
15    antibody to blood cells and only background levels  
16    of radiolabel were seen on endothelium or normal  
17    tissues. The antigen recognised by 791T/36 was  
18    recently identified as CD55 (Spendlove et al Eur J  
19    Immunol. 30:2944-2953; Spendlove et al Cancer Res.  
20    59:2282-2286). Using CD55/CD46 chimeric constructs  
21    it was possible to map the binding site of 791T/36  
22    to the first two SCR domains of CD55 with peptide  
23    analysis showing that 791T/36 can bind to three  
24    distinct regions of SCR1-2 of CD55. One region is in  
25    SCR1 and two are in SCR2.

26

27    WO00/5204 discloses a method for making antibodies,  
28    for example antibodies directed against decay  
29    accelerating factor (DAF, using a naïve antibody  
30    phage library. Although the document refers to the  
31    use of such antibodies in cancer diagnosis or  
32    therapy, no examples are provided other than a

1 speculative example, in which antibody LU30 is  
2 suggested for use in assessing overexpression of DAF  
3 and for treatment of lung cancer particularly when  
4 combined with cytotoxic agents.

5

6 WO/04415 describes the production of the anti-  
7 idiotype antibody 105AD7 which was raised against  
8 antibody 791T/36 and speculates on potential  
9 therapeutic uses of the 105AD7 antibody.

10

11 However, to date, no therapeutically useful anti-  
12 CD55 antibodies other than anti SCR3 antibodies have  
13 been demonstrated. Therapeutic studies with  
14 antibodies directed to other SCRs of this molecule  
15 have been limited to immunoconjugated molecules.

16 (See for example US 4916213 (Xoma Corporation), US  
17 4925922 (Xoma Corporation) and Byers et al. 1987  
18 Cancer Res 47:5042-5046). For example, Byers et al  
19 describes studies with 791T/36 linked to ricin A  
20 chain, showed significantly inhibition of tumour  
21 growth in athymic mice. 791T/36-RTA was therefore  
22 screened in a phase I clinical trial in advanced  
23 colorectal cancer patients (Byers et al 1989. Cancer  
24 Research 49:6153-6160). However the trial was  
25 unsuccessful due to dose limiting toxicity.

26

27 Surprisingly, the present inventors have now  
28 demonstrated that, although previous studies have  
29 demonstrated that antibodies which target either SCR  
30 1 or SCR 2 of CD55 failed to have any neutralisation  
31 effect on CD55, an antibody which targets both SCR 1

1 and SCR2 not only effectively neutralises CD55 but  
2 is superior to a SCR3 neutralising antibody.

3

4 Accordingly, in a first aspect, the present  
5 invention provides a method of neutralisation of  
6 CD55, comprising administration of a naked binding  
7 member which specifically binds to SCR1 and SCR2 of  
8 CD55.

9

10 By neutralising CD55, enhanced complement deposition  
11 may be facilitated. Accordingly, in a second aspect,  
12 the invention provides a method of enhancing  
13 complement deposition on a tissue comprising  
14 administration of a naked binding member which  
15 specifically binds to SCR1 and SCR2 of CD55.

16

17 The methods of the invention may be used *in vitro* or  
18 *in vivo*.

19

20 As described above, CD55 is commonly found on many  
21 tumour cell surfaces, where it serves to inhibit  
22 complement deposition. By neutralising such  
23 molecules on tumour cells, the methods of the  
24 invention enable complement mediated attack of  
25 tumour cells. Accordingly, in a further aspect of  
26 the present invention, there is provided a method of  
27 treating cancer comprising administration of a  
28 therapeutically effective amount of a naked binding  
29 member which specifically binds to SCR1 and SCR2 of  
30 CD55 to a mammal in need thereof.

31

1 In a further aspect, there is provided the use of  
2 (i) a naked binding member which binds to both SCR1  
3 and SCR2 of CD55 or (ii) a nucleic acid encoding  
4 said binding member in the preparation of a  
5 medicament for the neutralisation of CD55.

6

7 In a further aspect, there is provided a naked  
8 binding member which binds to both SCR1 and SCR2 for  
9 use in the treatment of cancer.

10

11 In a further aspect, there is provided the use of  
12 (i) a naked binding member which binds to both SCR1  
13 and SCR2 of CD55 or (ii) a nucleic acid encoding  
14 said binding member in the preparation of a  
15 medicament for treating cancer.

16

17 The present invention also provides a pharmaceutical  
18 composition for the treatment of cancer, wherein the  
19 composition comprises a naked binding member that  
20 binds to both SCR1 and SCR2 of CD55.

21

## 22 **Specific Binding Member**

23

24 As used herein, a "binding member" is a member of a  
25 pair of molecules which have binding specificity for  
26 one another. The binding member is, therefore, a  
27 specific binding member. The members of a binding  
28 pair may be naturally derived or wholly or partially  
29 synthetically produced. One member of the pair of  
30 molecules may have an area on its surface, which may  
31 be a protrusion or a cavity, which specifically  
32 binds to and is therefore complementary to a

1 particular spatial and polar organisation of the  
2 other member of the pair of molecules. Thus, the  
3 members of the pair have the property of binding  
4 specifically to each other. Examples of types of  
5 binding pairs are antigen-antibody, biotin-avidin,  
6 hormone-hormone receptor, receptor-ligand, enzyme-  
7 substrate. The present invention is concerned with  
8 antigen-antibody type reactions, although a binding  
9 member of the invention and for use in the invention  
10 may be any moiety which can bind to both SCR1 and  
11 SCR2 of CD55.

12

13 As used herein, "naked" means that the binding  
14 member of or for use in the present invention is not  
15 bound to, for example conjugated with, any agent,  
16 for example ricin, having anti-tumour properties.

17

#### 18 **Antibodies**

19

20 An "antibody" is an immunoglobulin, whether natural  
21 or partly or wholly synthetically produced. The  
22 term also covers any polypeptide, protein or peptide  
23 having a binding domain which is, or is homologous  
24 to, an antibody binding domain. These can be  
25 derived from natural sources, or they may be partly  
26 or wholly synthetically produced. Examples of  
27 antibodies are the immunoglobulin isotypes and their  
28 isotypic subclasses and fragments which comprise an  
29 antigen binding domain such as Fab, scFv, Fv, dAb,  
30 Fd; and diabodies.

31

1 The binding member of the invention may be an  
2 antibody such as a monoclonal or polyclonal  
3 antibody, or a fragment thereof. The constant region  
4 of the antibody may be of any class including, but  
5 not limited to, human classes IgG, IgA, IgM, IgD and  
6 IgE. The antibody may belong to any sub class e.g.  
7 IgG1, IgG2, IgG3 and IgG4. IgG1 is preferred. In  
8 preferred embodiments the antibody is 791T/36  
9 produced by the cell line deposited with ATCC under  
10 accession no. HB9173.

11

12 As antibodies can be modified in a number of ways,  
13 the term "antibody" should be construed as covering  
14 any binding member or substance having a binding  
15 domain with the required specificity. Thus, this  
16 term covers antibody fragments, derivatives,  
17 functional equivalents and homologues of antibodies,  
18 including any polypeptide comprising an  
19 immunoglobulin binding domain, whether natural or  
20 wholly or partially synthetic. Chimeric molecules  
21 comprising an immunoglobulin binding domain, or  
22 equivalent, fused to another polypeptide are  
23 therefore included. Cloning and expression of  
24 chimeric antibodies are described in EP-A-0120694  
25 and EP-A-0125023.

26

27 It has been shown that fragments of a whole antibody  
28 can perform the function of binding antigens.  
29 Examples of such binding fragments are (i) the Fab  
30 fragment consisting of VL, VH, CL and CH1 domains;  
31 (ii) the Fd fragment consisting of the VH and CH1  
32 domains; (iii) the Fv fragment consisting of the VL

1 and VH domains of a single antibody; (iv) the dAb  
2 fragment (Ward, E.S. et al., *Nature* 341:544-546  
3 (1989)) which consists of a VH domain; (v) isolated  
4 CDR regions; (vi) F(ab')<sub>2</sub> fragments, a bivalent  
5 fragment comprising two linked Fab fragments (vii)  
6 single chain Fv molecules (scFv), wherein a VH  
7 domain and a VL domain are linked by a peptide  
8 linker which allows the two domains to associate to  
9 form an antigen binding site (Bird et al., *Science*  
10 242:423-426 (1988); Huston et al., *PNAS USA* 85:5879-  
11 5883 (1988)); (viii) bispecific single chain Fv  
12 dimers (PCT/US92/09965) and (ix) "diabodies",  
13 multivalent or multispecific fragments constructed  
14 by gene fusion (WO94/13804; P. Hollinger et al.,  
15 *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993)).

16  
17 A fragment of an antibody or of a polypeptide for  
18 use in the present invention, for example, a  
19 fragment of the 791T/36 antibody, generally means a  
20 stretch of amino acid residues of at least 5 to 7  
21 contiguous amino acids, often at least about 7 to 9  
22 contiguous amino acids, typically at least about 9  
23 to 13 contiguous amino acids, more preferably at  
24 least about 20 to 30 or more contiguous amino acids  
25 and most preferably at least about 30 to 40 or more  
26 consecutive amino acids. A preferred group of  
27 fragments are those which include all or part of the  
28 CDR regions of monoclonal antibody 791T/36. A  
29 preferred group of fragments are those which include  
30 all or part of the CDR regions of monoclonal  
31 antibody 791T/36.

32

1 A "derivative" of such an antibody or polypeptide,  
2 or of a fragment of a 791T/36 antibody means an  
3 antibody or polypeptide modified by varying the  
4 amino acid sequence of the protein, e.g. by  
5 manipulation of the nucleic acid encoding the  
6 protein or by altering the protein itself. Such  
7 derivatives of the natural amino acid sequence may  
8 involve insertion, addition, deletion and/or  
9 substitution of one or more amino acids, preferably  
10 while providing a peptide having anti-CD55 activity,  
11 for example, CD55 neutralisation activity.

12 Preferably such derivatives involve the insertion,  
13 addition, deletion and/or substitution of 25 or  
14 fewer amino acids, more preferably of 15 or fewer,  
15 even more preferably of 10 or fewer, more preferably  
16 still of 4 or fewer and most preferably of 1 or 2  
17 amino acids only.

18  
19 The term "antibody" includes antibodies which have  
20 been "humanised". Methods for making humanised  
21 antibodies are known in the art. Methods are  
22 described, for example, in Winter, U.S. Patent No.  
23 5,225,539. A humanised antibody may be a modified  
24 antibody having the hypervariable region of a  
25 monoclonal antibody such as 791T/36 and the constant  
26 region of a human antibody. Thus the binding member  
27 may comprise a human constant region.

28  
29 The variable region other than the hypervariable  
30 region may also be derived from the variable region  
31 of a human antibody and/or may also be derived from  
32 a monoclonal antibody such as 791T/36. In such



1 case, the entire variable region may be derived from  
2 murine monoclonal antibody 791T/36 and the antibody  
3 is said to be chimerised. Methods for making  
4 chimerised antibodies are known in the art. Such  
5 methods include, for example, those described in  
6 U.S. patents by Boss (Celltech) and by Cabilly  
7 (Genentech). See U.S. Patent Nos. 4,816,397 and  
8 4,816,567, respectively.

9  
10 It is possible to take monoclonal and other  
11 antibodies and use techniques of recombinant DNA  
12 technology to produce other antibodies or chimeric  
13 molecules which retain the specificity of the  
14 original antibody. Such techniques may involve  
15 introducing DNA encoding the immunoglobulin variable  
16 region, or the complementary determining regions  
17 (CDRs), of an antibody to the constant regions, or  
18 constant regions plus framework regions, of a  
19 different immunoglobulin. See, for instance, EP-A-  
20 184187, GB 2188638A or EP-A-239400. A hybridoma or  
21 other cell producing an antibody may be subject to  
22 genetic mutation or other changes, which may or may  
23 not alter the binding specificity of antibodies  
24 produced.

25  
26 In preferred embodiments of the invention, the  
27 binding member binds to CD55 SCR1 (amino acids 83-  
28 93) and SCR2 (amino acids 101-112 and amino acids  
29 145-157) of the sequences shown in Figure 1b.

30  
31 The binding member may comprise one or more of the  
32 CDRs of the antibody, or a fragment thereof,

1 produced by the cell line deposited at ATCC under  
2 accession number HB9173.

3

4 As described above, in a preferred embodiment of the  
5 invention, the binding member is the antibody  
6 791T/36 produced by the hybridoma cell deposited  
7 under ATCC accession number HB9173. As used herein,  
8 reference to "791T/36" includes sequences which show  
9 substantial homology with 791T/36. Preferably the  
10 degree of homology between 791T/36 complementary  
11 determining regions (CDRs) and the CDRs of other  
12 antibodies will be at least 60%, more preferably  
13 70%, further preferably 80%, even more preferably  
14 90% or most preferably 95%.

15

16 The percent identity of two amino acid sequences or  
17 of two nucleic acid sequences may be determined by  
18 aligning the sequences for optimal comparison  
19 purposes (e.g., gaps can be introduced in the first  
20 sequence for best alignment with the sequence) and  
21 comparing the amino acid residues or nucleotides at  
22 corresponding positions. The "best alignment" is an  
23 alignment of two sequences which results in the  
24 highest percent identity. The percent identity is  
25 determined by the number of identical amino acid  
26 residues or nucleotides in the sequences being  
27 compared (i.e., % identity = number of identical  
28 positions/total number of positions x 100).

29

30 The determination of percent identity between two  
31 sequences can be accomplished using a mathematical  
32 algorithm known to those of skill in the art. An

1     example of a mathematical algorithm for comparing  
2     two sequences is the algorithm of Karlin and  
3     Altschul (1990) *Proc. Natl. Acad. Sci. USA*  
4     87:2264-2268, modified as in Karlin and Altschul  
5     (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The  
6     NBLAST and XBLAST programs of Altschul, *et al.*  
7     (1990) *J. Mol. Biol.* 215:403-410 have incorporated  
8     such an algorithm. BLAST nucleotide searches can be  
9     performed with the NBLAST program, score = 100,  
10    wordlength = 12 to obtain nucleotide sequences  
11    homologous to nucleic acid molecules of the  
12    invention. BLAST protein searches can be performed  
13    with the XBLAST program, score = 50, wordlength = 3  
14    to obtain amino acid sequences homologous to protein  
15    molecules of the invention. To obtain gapped  
16    alignments for comparison purposes, Gapped BLAST can  
17    be utilised as described in Altschul *et al.* (1997)  
18    *Nucleic Acids Res.* 25:3389-3402. Alternatively,  
19    PSI-Blast can be used to perform an iterated search  
20    which detects distant relationships between  
21    molecules (*Id.*). When utilising BLAST, Gapped  
22    BLAST, and PSI-Blast programs, the default  
23    parameters of the respective programs (e.g., XBLAST  
24    and NBLAST) can be used. See  
25    <http://www.ncbi.nlm.nih.gov>.

26  
27    Another example of a mathematical algorithm utilised  
28    for the comparison of sequences is the algorithm of  
29    Myers & Miller, CABIOS (1989). The ALIGN program  
30    (version 2.0) which is part of the CGC sequence  
31    alignment software package has incorporated such an  
32    algorithm. Other algorithms for sequence analysis

1 known in the art include ADVANCE and ADAM as  
2 described in Torellis & Robotti (1994) *Comput. Appl.*  
3 *Biosci.*, 10 :3-5; and FASTA described in Pearson &  
4 Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-8.  
5 Within FASTA, ktup is a control option that sets the  
6 sensitivity and speed of the search.

7  
8 Where high degrees of sequence identity are present  
9 there will be relatively few differences in amino  
10 acid sequence. Thus for example they may be less  
11 than 20, less than 10, or even less than 5  
12 differences.

13  
14 The present inventors have shown that antibodies  
15 directed to SCR1 and SCR2 of CD55, for example  
16 791T/36 antibodies and fragments and derivatives  
17 thereof can be used as cancer therapeutics to  
18 inactivate CD55 and make tumour cells susceptible to  
19 complement mediated attack. This is exemplified by  
20 localisation of the antibody within tumours of  
21 cancer patients and their subsequent enhanced  
22 survival (see the Examples). Accordingly the  
23 invention further provides the use of naked  
24 "fragments" or "derivatives" of 791T/36 or other  
25 polypeptides of the "791T/36" family which bind to  
26 both SCR1 and SCR2 CD55 epitopes in the preparation  
27 of an agent for treating cancer.

28  
29 The binding members may be administered alone or in  
30 combination with one or more further agents. Thus,  
31 the present invention further provides products  
32 comprising a naked binding member, which binds to

1 both SCR1 and SCR2 of CD55, and an active agent as a  
2 combined preparation for simultaneous, separate or  
3 sequential use in the treatment of cancer. Active  
4 agents may include chemotherapeutic agents  
5 including, Doxorubicin, taxol, 5-Fluorouracil (5  
6 FU), Leucovorin, Irinotecan, Mitomycin C,  
7 Oxaliplatin, Raltitrexed, Tamoxifen and Cisplatin  
8 which may operate synergistically with the binding  
9 member of the present invention. Other active agents  
10 may include suitable doses of pain relief drugs such  
11 as non-steroidal anti-inflammatory drugs (e.g.  
12 aspirin, paracetamol, ibuprofen or ketoprofen) or  
13 opiates such as morphine, or anti-emetics. In  
14 further embodiments, the active agent may be a  
15 further binding member. Thus, in preferred  
16 embodiments the binding member may be administered  
17 in combination with one or more further binding  
18 members. Such binding members may include but are  
19 not limited to an anti-CD20 antibody e.g. Rituxan  
20 (Rituximab) (Biogen IDEC (Cambridge, MA, USA); an  
21 anti-VEGF antibody e.g. Avastin (bevacizumab),  
22 Genentech (South San Francisco, CA, USA) / Roche  
23 (Basel, Switzerland); an anti-CD171A antibody, e.g.  
24 Panorex (edrecolomab) Centocor (Malvern, PA, USA) /  
25 Glaxo SmithKline (Uxbridge, UK); an anti-CEA anti-  
26 idiotypic mAb e.g. CeaVac, Titan Pharmaceuticals  
27 (South San Francisco, CA, USA); an anti-EGFR  
28 antibody e.g. Erbitux (cetuximab), ImClone (New York,  
29 USA) / Bristol Myers Squibb (New York, USA), Merck  
30 (Whitehouse Station, NJ, USA); an anti-HMFG anti-  
31 idiotypic mAb e.g. TriAb, Titan Pharmaceuticals  
32 (South San Francisco, CA, USA), an anti-EGFR

1     antibody e.g. ABX-EGF, Abgenix (Fremont, CA, USA)  
2     /Amgen Thousand Oaks, CA) and/or an anti-HER2  
3     antibody e.g. Herceptin, Genentech (South San  
4     Francisco, CA, USA).

5  
6     Preferably, the active agent synergises with the  
7     binding member. The ability of the binding member to  
8     synergise with an active agent to enhance tumour  
9     killing may not be due to immune effector mechanisms  
10    but rather may be a direct consequence of  
11    inactivating CD55 allowing enhanced complement  
12    deposition and complement lysis. The binding member  
13    of the invention may carry a detectable label.

14

#### 15     **Treatment**

16

17     "Treatment" includes any regime that can benefit a  
18     human or non-human animal. The treatment may be in  
19     respect of an existing condition or may be  
20     prophylactic (preventative treatment). Treatment may  
21     include curative, alleviation or prophylactic  
22     effects.

23

24     "Treatment of cancer" includes treatment of  
25     conditions caused by cancerous growth and includes  
26     the treatment of neoplastic growths or tumours.  
27     Examples of tumours that can be treated by the  
28     system of the invention are, for instance, sarcomas,  
29     including osteogenic and soft tissue sarcomas,  
30     carcinomas, e.g., breast-, lung-, bladder-, thyroid-  
31     , prostate-, colon-, rectum-, pancreas-, stomach-,  
32     liver-, uterine-, cervical and ovarian carcinoma,

1 lymphomas, including Hodgkin and non-Hodgkin  
2 lymphomas, neuroblastoma, melanoma, myeloma, Wilms  
3 tumor, and leukemias, including acute lymphoblastic  
4 leukaemia and acute myeloblastic leukaemia, gliomas  
5 and retinoblastomas.

6  
7 The binding member may, upon binding to SCR1 and  
8 SCR2 of CD55 present on cancerous cells or tissues,  
9 including tumour and non-tumour cells, neutralise  
10 CD55 and enhance complement deposition and  
11 complement mediated lysis of these cells.

12  
13 The compositions and methods of the invention may be  
14 particularly useful in the treatment of existing  
15 cancer and in the prevention of the recurrence of  
16 cancer after initial treatment or surgery.

17  
18 **Administration**

19  
20 Binding members of the present invention may be  
21 administered alone but will preferably be  
22 administered as a pharmaceutical composition, which  
23 will generally comprise a suitable pharmaceutical  
24 excipient, diluent or carrier selected dependent on  
25 the intended route of administration.

26 Binding members of the present invention may be  
27 administered to a patient in need of treatment via  
28 any suitable route. The precise dose will depend  
29 upon a number of factors, including the precise  
30 nature of the member (e.g. whole antibody, fragment  
31 or diabody), and the nature of the detectable label  
32 attached to the member.

1

2 Some suitable routes of administration include (but  
3 are not limited to) oral, rectal, nasal, topical  
4 (including buccal and sublingual), vaginal or  
5 parenteral (including subcutaneous, intramuscular,  
6 intravenous, intradermal, intrathecal and epidural)  
7 administration. Intravenous administration is  
8 preferred.

9

10 It is envisaged that injections (intravenous) will  
11 be the primary route for therapeutic administration  
12 of the compositions although delivery through a  
13 catheter or other surgical tubing is also envisaged.  
14 Liquid formulations may be utilised after  
15 reconstitution from powder formulations.

16

17 For intravenous, injection, or injection at the site  
18 of affliction, the active ingredient will be in the  
19 form of a parenterally acceptable aqueous solution  
20 which is pyrogen-free and has suitable pH,  
21 isotonicity and stability. Those of relevant skill  
22 in the art are well able to prepare suitable  
23 solutions using, for example, isotonic vehicles such  
24 as Sodium Chloride Injection, Ringer's Injection,  
25 Lactated Ringer's Injection. Preservatives,  
26 stabilisers, buffers, antioxidants and/or other  
27 additives may be included, as required.

28

29 Pharmaceutical compositions for oral administration  
30 may be in tablet, capsule, powder or liquid form. A  
31 tablet may comprise a solid carrier such as gelatin  
32 or an adjuvant. Liquid pharmaceutical compositions



1 generally comprise a liquid carrier such as water,  
2 petroleum, animal or vegetable oils, mineral oil or  
3 synthetic oil. Physiological saline solution,  
4 dextrose or other saccharide solution or glycols  
5 such as ethylene glycol, propylene glycol or  
6 polyethylene glycol may be included.

7  
8 The composition may also be administered via  
9 microspheres, liposomes, other microparticulate  
10 delivery systems or sustained release formulations  
11 placed in certain tissues including blood. Suitable  
12 examples of sustained release carriers include  
13 semipermeable polymer matrices in the form of shared  
14 articles, e.g. suppositories or microcapsules.  
15 Implantable or microcapsular sustained release  
16 matrices include polylactides (US Patent No. 3, 773,  
17 919; EP-A-0058481) copolymers of L-glutamic acid and  
18 gamma ethyl-L-glutamate (Sidman et al, Biopolymers  
19 22(1): 547-556, 1985), poly (2-hydroxyethyl-  
20 methacrylate) or ethylene vinyl acetate (Langer et  
21 al, J. Biomed. Mater. Res. 15: 167-277, 1981, and  
22 Langer, Chem. Tech. 12:98-105, 1982). Liposomes  
23 containing the polypeptides are prepared by well-  
24 known methods: DE 3,218, 121A; Epstein et al, PNAS  
25 USA, 82: 3688-3692, 1985; Hwang et al, PNAS USA, 77:  
26 4030-4034, 1980; EP-A-0052522; E-A-0036676; EP-A-  
27 0088046; EP-A-0143949; EP-A-0142541; JP-A-83-11808;  
28 US Patent Nos 4,485,045 and 4,544,545. Ordinarily,  
29 the liposomes are of the small (about 200-800  
30 Angstroms) unilamellar type in which the lipid  
31 content is greater than about 30 mol. % cholesterol,

1 the selected proportion being adjusted for the  
2 optimal rate of the polypeptide leakage.

3

4 Examples of the techniques and protocols mentioned  
5 above and other techniques and protocols which may  
6 be used in accordance with the invention can be  
7 found in Remington's Pharmaceutical Sciences, 16<sup>th</sup>  
8 edition, Oslo, A. (ed), 1980.

9

10 The composition may be administered in a localised  
11 manner to a tumour site or other desired site or may  
12 be delivered in a manner in which it targets tumour  
13 or other cells. Targeting therapies may be used to  
14 deliver the active agent more specifically to  
15 certain types of cell, by the use of targeting  
16 systems such as antibody or cell specific ligands.  
17 Targeting may be desirable for a variety of reasons,  
18 for example if the agent is unacceptably toxic, or  
19 if it would otherwise require too high a dosage, or  
20 if it would not otherwise be able to enter the  
21 target cells.

22

### 23 **Pharmaceutical Compositions**

24

25 As described above, the present invention extends to  
26 a pharmaceutical composition for the treatment of  
27 cancer, the composition comprising a naked binding  
28 member which binds to both SCR1 and SCR2 of CD55.  
29 Pharmaceutical compositions according to the present  
30 invention, and for use in accordance with the  
31 present invention may comprise, in addition to  
32 active ingredient, a pharmaceutically acceptable

1     excipient, carrier, buffer stabiliser or other  
2     materials well known to those skilled in the art.  
3     Such materials should be non-toxic and should not  
4     interfere with the efficacy of the active  
5     ingredient. The precise nature of the carrier or  
6     other material will depend on the route of  
7     administration, which may be oral, or by injection,  
8     e.g. intravenous.

9  
10    The formulation may be a liquid, for example, a  
11    physiologic salt solution containing non-phosphate  
12    buffer at pH 6.8-7.6, or a lyophilised powder.

13  
14    **Dose**

15  
16    The compositions are preferably administered to an  
17    individual in a "therapeutically effective amount",  
18    this being sufficient to show benefit to the  
19    individual. The actual amount administered, and  
20    rate and time-course of administration, will depend  
21    on the nature and severity of what is being treated.  
22    Prescription of treatment, e.g. decisions on dosage  
23    etc, is ultimately within the responsibility and at  
24    the discretion of general practitioners and other  
25    medical doctors, and typically takes account of the  
26    disorder to be treated, the condition of the  
27    individual patient, the site of delivery, the method  
28    of administration and other factors known to  
29    practitioners.

30  
31    The optimal dose can be determined by physicians  
32    based on a number of parameters including, for

1     example, age, sex, weight, severity of the condition  
2     being treated, the active ingredient being  
3     administered and the route of administration. In  
4     general, a serum concentration of polypeptides and  
5     antibodies that permits saturation of receptors is  
6     desirable. A concentration in excess of  
7     approximately 0.1nM is normally sufficient. For  
8     example, a dose of 100mg/m<sup>2</sup> of antibody provides a  
9     serum concentration of approximately 20nM for  
10    approximately eight days.

11  
12    As a rough guideline, doses of antibodies may be  
13    given weekly in amounts of 10-300mg/m<sup>2</sup>. Equivalent  
14    doses of antibody fragments should be used at more  
15    frequent intervals in order to maintain a serum  
16    level in excess of the concentration that permits  
17    saturation of CD55.

#### 18 19    **Production of Binding Members**

20  
21    The binding members of and for use in the present  
22    invention may be generated wholly or partly by  
23    chemical synthesis. The binding members can be  
24    readily prepared according to well-established,  
25    standard liquid or, preferably, solid-phase peptide  
26    synthesis methods, general descriptions of which are  
27    broadly available (see, for example, in J.M. Stewart  
28    and J.D. Young, Solid Phase Peptide Synthesis, 2<sup>nd</sup>  
29    edition, Pierce Chemical Company, Rockford, Illinois  
30    (1984), in M. Bodanzsky and A. Bodanzsky, The  
31    Practice of Peptide Synthesis, Springer Verlag, New  
32    York (1984); and Applied Biosystems 430A Users

1 Manual, ABI Inc., Foster City, California), or they  
2 may be prepared in solution, by the liquid phase  
3 method or by any combination of solid-phase, liquid  
4 phase and solution chemistry, e.g. by first  
5 completing the respective peptide portion and then,  
6 if desired and appropriate, after removal of any  
7 protecting groups being present, by introduction of  
8 the residue X by reaction of the respective carbonic  
9 or sulfonic acid or a reactive derivative thereof.

10

11 Another convenient way of producing a binding member  
12 suitable for use in the present invention is to  
13 express nucleic acid encoding it, by use of nucleic  
14 acid in an expression system. Thus the present  
15 invention further provides the use of an isolated  
16 nucleic acid encoding a naked binding member which  
17 binds to both SCR1 and SCR2 of CD55 in the  
18 preparation of an agent for treating cancer.

19

20 Nucleic acid for use in accordance with the present  
21 invention may comprise DNA or RNA and may be wholly  
22 or partially synthetic. In a preferred aspect,  
23 nucleic acid for use in the invention codes for a  
24 binding member of the invention as defined above.  
25 The skilled person will be able to determine  
26 substitutions, deletions and/or additions to such  
27 nucleic acids which will still provide a binding  
28 member of the present invention.

29

30 Nucleic acid sequences encoding a binding member for  
31 use with the present invention can be readily  
32 prepared by the skilled person using the information

1 and references contained herein and techniques known  
2 in the art (for example, see Sambrook, Fritsch and  
3 Maniatis, "Molecular Cloning", A Laboratory Manual,  
4 Cold Spring Harbor Laboratory Press, 1989, and  
5 Ausubel et al, Short Protocols in Molecular Biology,  
6 John Wiley and Sons, 1992), given the nucleic acid  
7 sequences and clones available. These techniques  
8 include (i) the use of the polymerase chain reaction  
9 (PCR) to amplify samples of such nucleic acid, e.g.  
10 from genomic sources, (ii) chemical synthesis, or  
11 (iii) preparing cDNA sequences. DNA encoding  
12 antibody fragments may be generated and used in any  
13 suitable way known to those of skill in the art,  
14 including by taking encoding DNA, identifying  
15 suitable restriction enzyme recognition sites either  
16 side of the portion to be expressed, and cutting out  
17 said portion from the DNA. The portion may then be  
18 operably linked to a suitable promoter in a standard  
19 commercially available expression system. Another  
20 recombinant approach is to amplify the relevant  
21 portion of the DNA with suitable PCR primers.  
22 Modifications to the sequences can be made, e.g.  
23 using site directed mutagenesis, to lead to the  
24 expression of modified peptide or to take account of  
25 codon preferences in the host cells used to express  
26 the nucleic acid.

27

28 The nucleic acid may be comprised as constructs in  
29 the form of a plasmid, vector, transcription or  
30 expression cassette which comprises at least one  
31 nucleic acid as described above. The construct may  
32 be comprised within a recombinant host cell which

1 comprises one or more constructs as above.  
2 Expression may conveniently be achieved by culturing  
3 under appropriate conditions recombinant host cells  
4 containing the nucleic acid. Following production  
5 by expression a specific binding member may be  
6 isolated and/or purified using any suitable  
7 technique, then used as appropriate.

8  
9 Binding members-encoding nucleic acid molecules and  
10 vectors for use in accordance with the present  
11 invention may be provided isolated and/or purified,  
12 e.g. from their natural environment, in  
13 substantially pure or homogeneous form, or, in the  
14 case of nucleic acid, free or substantially free of  
15 nucleic acid or genes origin other than the sequence  
16 encoding a polypeptide with the required function.

17  
18 Systems for cloning and expression of a polypeptide  
19 in a variety of different host cells are well known.  
20 Suitable host cells include bacteria, mammalian  
21 cells, yeast and baculovirus systems. Mammalian  
22 cell lines available in the art for expression of a  
23 heterologous polypeptide include Chinese hamster  
24 ovary cells, HeLa cells, baby hamster kidney cells,  
25 NSO mouse melanoma cells and many others. A common,  
26 preferred bacterial host is *E. coli*.

27  
28 The expression of antibodies and antibody fragments  
29 in prokaryotic cells such as *E. coli* is well  
30 established in the art. For a review, see for  
31 example Plückthun, *Bio/Technology* 9:545-551 (1991).  
32 Expression in eukaryotic cells in culture is also

1 available to those skilled in the art as an option  
2 for production of a binding member, see for recent  
3 review, for example Reff, *Curr. Opinion Biotech.*  
4 4:573-576 (1993); Trill et al., *Curr. Opinion*  
5 *Biotech.* 6:553-560 (1995).

6  
7 ALternatively, the specific binding members for use  
8 in the invention may be produced in transgenic  
9 organisms, for example mammals, avians, fish,  
10 insects or plants using methods known in the art. In  
11 such transgenic methods, nucleic acid encoding the  
12 binding member(s) may be introduced to the cell or  
13 embryo by methods including but not limited to  
14 direct injection, electroporation, nuclear transfer  
15 techniques or by use of vectors, e.g. viral vectors.  
16 In one preferred embodiment, the specific binding  
17 members are produced in avian tissues, preferably  
18 avian eggs, using, for example, the method as  
19 disclosed in GB 0227645.9, filed 27 November 2002  
20 and the subsequent PCT application claiming priority  
21 therefrom.

22  
23 Suitable vectors can be chosen or constructed,  
24 containing appropriate regulatory sequences,  
25 including promoter sequences, terminator sequences,  
26 polyadenylation sequences, enhancer sequences,  
27 marker genes and other sequences as appropriate.  
28 Vectors may be plasmids, viral e.g. 'phage, or  
29 phagemid, as appropriate. For further details see,  
30 for example, Sambrook et al., *Molecular Cloning: A*  
31 *Laboratory Manual*: 2<sup>nd</sup> Edition, Cold Spring Harbor  
32 Laboratory Press (1989). Many known techniques and



1 protocols for manipulation of nucleic acid, for  
2 example in preparation of nucleic acid constructs,  
3 mutagenesis, sequencing, introduction of DNA into  
4 cells and gene expression, and analysis of proteins,  
5 are described in detail in Ausubel et al. eds.,  
6 *Short Protocols in Molecular Biology*, 2<sup>nd</sup> Edition,  
7 John Wiley & Sons (1992).

8

9 The nucleic acid may be introduced into a host cell  
10 by any suitable means. The introduction may employ  
11 any available technique. For eukaryotic cells,  
12 suitable techniques may include calcium phosphate  
13 transfection, DEAE-Dextran, electroporation,  
14 liposome-mediated transfection and transduction  
15 using retrovirus or other virus, e.g. vaccinia or,  
16 for insect cells, baculovirus. For bacterial cells,  
17 suitable techniques may include calcium chloride  
18 transformation, electroporation and transfection  
19 using bacteriophage.

20

21 Marker genes such as antibiotic resistance or  
22 sensitivity genes may be used in identifying clones  
23 containing nucleic acid of interest, as is well  
24 known in the art.

25

26 The introduction may be followed by causing or  
27 allowing expression from the nucleic acid, e.g. by  
28 culturing host cells under conditions for expression  
29 of the gene.

30

31 The nucleic acid may be integrated into the genome  
32 (e.g. chromosome) of the host cell. Integration may

1 be promoted by inclusion of sequences which promote  
2 recombination with the genome in accordance with  
3 standard techniques. The nucleic acid may be on an  
4 extra-chromosomal vector within the cell, or  
5 otherwise identifiably heterologous or foreign to  
6 the cell.

7

#### 8 **Assays**

9

10 The invention further provides assays for  
11 identification of further agents, for example  
12 antibodies that can be used for the enhancement of  
13 complement deposition on a cell sample or tissue and  
14 which can optionally be used in the treatment of  
15 cancer.

16

17 In a preferred aspect, the assay comprises an assay  
18 method for identification of an agent capable of  
19 inhibiting CD55 comprising steps:

20

- 21 a) bringing into contact a candidate agent with at  
22 least a portion of SCR1 and SCR2 of CD55; and  
23  
24 b) determining binding of said candidate agent to  
25 both SCR1 and SCR2.

26

27 In a further embodiment, the assay method comprises  
28 a method for identification of an agent capable of  
29 inhibiting CD55 comprising:

30

- 31 (a) bringing into contact a candidate agent with at  
32 least a portion of SCR1 and SCR2 of CD55 in the

1           presence of a naked binding member which in the  
2           absence of the candidate agent is capable of  
3           binding both SCR1 and SCR2 of CD55; and  
4

5       (b) determining the extent to which the candidate  
6           agent inhibits binding of the naked binding  
7           member to SCR1 and SCR2 of CD55.  
8

9       The assays may further comprise the step of  
10       selecting a candidate agent which binds both SCR1  
11       and SCR2 of CD55; and/or the step of determining  
12       the amount of complement deposition on a cell sample  
13       in the presence and absence of the candidate agent.  
14

15       In preferred embodiments of the assays of the  
16       invention, the portion of SCR1 and SCR2 of CD55  
17       comprises amino acids 83-93, 101-112 and 145-157 of  
18       the sequences shown in Figure 1b.  
19

20       The present invention further provides a screening  
21       method comprising the step of screening a library of  
22       candidate agents for the ability to inhibit the  
23       binding of a naked binding member to both SCR1 and  
24       SCR2 of CD55.  
25

26       The assay of the invention may be a screen , whereby  
27       a number of candidate agents are tested.

28       Accordingly, any suitable technique for screening  
29       compounds known to the person skilled in the art may  
30       be used. The screen may be a high-throughput  
31       screen. For example, WO84/03564 describes a method  
32       in which large numbers of peptides are synthesised

1 on a solid substrate and reacted with an agent and  
2 washed. Bound entities are detected.

3

4 The invention also contemplates the use of  
5 competitive drug screening assays in which  
6 neutralising antibodies such as 791T/36 capable of  
7 binding SCR1 and 2 of CD55 specifically compete with  
8 a test compound for binding to SCR1 and 2 of CD55.

9

10 Agents identified by the screening method of the  
11 present invention and their use in the manufacture  
12 of a medicament for the treatment of cancer are also  
13 contemplated by the invention.

14

15 Preferred features of each aspect of the invention  
16 are as for each of the other aspects *mutatis*  
17 *mutandis*.

18

19 The invention will now be described further in the  
20 following non-limiting examples. Reference is made  
21 to the accompanying drawings in which:

22

23 Figure 1a represents the translated CDR sequences of  
24 VK and VH cDNAs from 105AD7 hybridoma. Uppercase  
25 letters represent the CDR regions, the lower case  
26 letters are the adjacent framework amino acids.

27

28 Figure 1b shows alignment of the three CDR peptides  
29 with CD55. The amino acid numbering is taken from  
30 the full-length sequence of CD55 including the  
31 leader sequence. CD55 peptides used in subsequent  
32 assays are shown underlined. Bullets (•) represent

1 amino acid identity whereas amino acids with similar  
2 physicochemical properties are marked as (|).

3

4 Figure 2 illustrates a C3b complement deposition  
5 assay. 791T cells were incubated with human serum as  
6 a source of complement. C3b deposition was measured  
7 using rabbit anti-C3b FITC labelled antibody in the  
8 presence of blocking (216), non blocking (220) or  
9 test antibody 791T/36. Fluorescence was quantified  
10 by a FACScan flow cytometer and is present as mean  
11 linear fluorescence (MLF).

#### 12 Example 1 CD55 Neutralisation Assay

13

14 Purified CD55 antigen was obtained by  
15 immunoaffinity-matrix purification from octyl-  
16 glucoside-solubilised 791T cells. CD55 cDNA was  
17 cloned and sequenced using primers based on protein  
18 sequence data obtained from the purified antigen  
19 (Spendlove et al., 1999 Cancer Res 59, 2282). The  
20 DNA sequence obtained was identical to that  
21 identified by Caras et al and present on the Genbank  
22 database (Accession No. M31516).

23

#### 24 **Cells**

25

26 791T is an osteosarcoma cell line which was grown in  
27 RPMI (Gibco, BRL, Paisley, and UK) supplemented with  
28 10% heat inactivated fetal calf serum.

29

#### 30 **Monoclonal Antibodies**

31

1 Monoclonal antibodies 791T/36 (IgG2b anti-791Tgp72;  
2 Embleton et al 1981 Br. J. Cancer 43:582-587), BRIC  
3 216 (IgG1 anti-SCR 3 of CD55; Tate et al 1989  
4 Biochem J 261, 489), BRIC 220 (IgG1 anti-SCR 1 of  
5 CD55, Tate et al 1989 Biochem J 261, 489), BRIC 110  
6 (IgG1 anti-SCR 2 of CD55; Spring et al., 1987  
7 Immunology 62 377; Coyne et al, 1992 J Immunol 149,  
8 2906) have been reported previously. The BRIC  
9 antibodies were purchased from the Blood Group  
10 Reference laboratory (Bristol, UK).  
11  
12

### 13 **Methods**

14

15 791T tumour cells that over-express CD55 were washed  
16 with media containing 10% FCS and resuspended at a  
17 density of  $1 \times 10^5$  cells per 100  $\mu$ l. Primary antibody  
18 was incubated with 3x sample volume ( $3 \times 10^5$   
19 cells/300  $\mu$ l) at a concentration of 50  $\mu$ g/ml. Primary  
20 antibodies were positive control antibody, 216  
21 (anti-SCR3), negative control antibody 220 (anti-  
22 SCR1) and test antibody, 791T/36 (anti-SCR1 and 2).  
23 Cells and antibodies were incubated for 1 hr at 4°C  
24 prior to washing in PBS. Samples were split into 3  
25 samples of 100  $\mu$ l per tube. Human Serum was added as  
26 a source of complement to total concentration of 5%  
27 (Not Heat Inactivated). Tubes were inverted several  
28 times and incubate at 37°C for 2 hours, mixing every  
29 30 min. Cells were washed twice in PBS prior to  
30 addition of polyclonal rabbit anti human C3c FITC  
31 conjugated antibody (1/100) to a final volume of  
32 100  $\mu$ l. Cells were incubated for 1 hour at 4°C prior

1 to washing twice in PBS and resuspending in 200µl of  
2 1% cell fix.

3

#### 4 **Results**

5

6 Figure 2 shows that in the presence of a non-  
7 blocking antibody 220 C3b is deposited onto 791T  
8 cells at modest levels (MLF 200). In the presence of  
9 the CD55 neutralising antibody, 216, enhanced C3b  
10 deposition is observed (MLF 350). However in the  
11 presence of monoclonal antibody 791T/36 even greater  
12 levels of C3b are deposited (MLF520). This suggests  
13 that although 216 is an effective competitor with C3  
14 convertase for binding to SCR3. binding of 791T/36  
15 to SCR1 and SCR2 domains functionally inactivates  
16 CD55 leading to a 250% increase in C3b deposition.

17

18 **Example 2. Long term survival of recurrent**  
19 **colorectal cancer patients receiving radiolabelled**  
20 **791T/36 for tumour imaging.**

21

#### 22 **Antibody and Labelling**

23

24 Hybridoma 791T/36 clone 3 is the source of antibody  
25 (791T/36, IgG2b isotype). Ascitic fluid from mice  
26 in which the hybridoma was developing was applied to  
27 a protein A-"Sepharose" column in pH 7.5 0.1 mol/l  
28 citrate phosphate buffer and the column was  
29 thoroughly washed. Bound immunoglobulins were  
30 eluted stepwise at pH 6.0, 5.0, 4.5 and 3.0 and  
31 these were then dialysed against phosphate-buffered  
32 saline. The dialysate was then centrifuged at

1 1000000g for 1 h, filtered through a 0.22µm Millex  
2 "Millipore" filter, and stored at -70°C at a protein  
3 concentration of 1mg/ml. The preparation contained  
4 only IgG2b as assessed by immunodiffusion tests with  
5 mouse immunoglobulin typing antisera (Miles  
6 Laboratories, Stoke Poges, Bucks.) and was pyrogen-  
7 free (Boots Pharmaceuticals, Notts).

8  
9 Batches of the antibody preparation were labelled  
10 with <sup>131</sup>I by means of "Iodogen" reagent. Non-bound  
11 iodine was removed by gel filtration on sephadex  
12 G25. Labelled preparations were diluted into saline  
13 containing 1% serum albumin and sterilised by Millex  
14 filtration.

15  
16 72 patients with recurrent colorectal cancer were  
17 imaged with the radiolabelled monoclonal antibody  
18 791T/36. Patients received an id dose of 10µg of  
19 antibody followed by an intravenous dose of 200µg.  
20 2dl of preparation containing 200µg of antibody and  
21 approximately 70MBq <sup>131</sup>I was infused into an  
22 antecubital vein of each patient over 30 min.

23  
24 Survival was followed for 7 years and compared to a  
25 contemporary group of recurrent colorectal cancer  
26 patients. There were 12 long term survivors (16%)  
27 in the patients who had received 791T/36 where as in  
28 contrast only 1 out of 89 patients survived 7 years  
29 in the contemporary group (p> 0.001).

30  
31 Table 1: Survival of colorectal cancer patients  
32 receiving 791T/36 antibody.



1

Patients	Survival	Death
Imaged with 791T/36	12	60
Contemporary controls	1	88

2

3 These results suggest that there is an apparent  
4 survival benefit in a non-randomised trial of  
5 patients receiving radiolabelled 791T/36 antibody.  
6 The dose of radiolabel reaching the tumour is well  
7 below the level required to elicit tumour killing as  
8 a result of the radiolabel alone. It is therefore  
9 more likely that the antibody is inactivating CD55,  
10 allowing complement attack of residual tumour. As  
11 these patients only received a single intravenous  
12 dose of 791T/36 antibody the apparent survival  
13 benefit is very dramatic. Repeat injection with a  
14 humanised 791T/36 antibody may have an even more  
15 pronounced therapeutic benefit.

16

17 **Example 3. Production of new monoclonal antibodies**  
18 **to SCR1 and SCR2**

19

20 6-8 week old Balb/c mice were immunised twice 3  
21 weeks apart by intraperitoneal injection with 791T  
22 cells that over-express CD55 antigen ( $10^6$  cells).  
23 Mice were then boosted with SCR1-2 protein fused to  
24 human Fc and purified by protein A chromatography.  
25 Mice were tail bled and serum was screened for their  
26 ability to recognise CD55SCR1-2/CD46SCR3-4 chimeric  
27 molecules expressed by CHO cells as previously  
28 described (Spendlove et al 2000 Eur J Immunol 30,  
29 2944). They were also screened for their ability to

1 recognise the SCR1-2CD55Fc protein and the IC, 2N  
2 and 2C peptides attached to BSA as previously  
3 described (Spendlove et al 2000 Eur J Immunol 30,  
4 2944). Mice producing antibodies that recognises  
5 CD55SCR1 and SCR2 are boosted by an intravenous  
6 injection of SCR1-2Fc protein and  
7 splenocytes removed 5 days later and fused using PEG  
8 with NSO myeloma cells at a 10:1 ratio. Hybridomas  
9 are selected using HAT medium and screened for  
10 production of antibodies recognising SRR1-2Fc  
11 protein by ELISA. Hybridomas producing the correct  
12 antibody are cloned by limiting dilution three times  
13 a 1 cells per well to ensure clonality. The  
14 monoclonal antibody is screened for its ability to  
15 recognise CD55SCR1-2/CD46SCR3-4 chimaeric molecules  
16 expressed by CHO cells as previously described  
17 (Spendlove et al 2000 Eur J Immunol 30, 2944). They  
18 are also screened for their ability to recognise the  
19 SCR1-2CD55Fc protein and the IC, 2N and 2C peptides  
20 attached to BSA as previously described (Spendlove  
21 et al 2000 Eur J Immunol 30, 2944). To determine if  
22 they recognise the same site as 791T/36 plates are  
23 coated with CD55 as described above. They are then  
24 incubated with the new monoclonal antibodies and  
25 then with biotinylated 791T/36. Binding of 791T/36  
26 is quantified by avidin peroxidase and ABTS  
27 substrate and the OD read at 405nm on a plate  
28 reader. If the monoclonal antibodies recognise the  
29 same or related sites to 791T/36 they will inhibit  
30 binding of 791T/36 to CD55 antigen.

1 All documents referred to in this specification are  
2 herein incorporated by reference. Various  
3 modifications and variations to the described  
4 embodiments of the inventions will be apparent to  
5 those skilled in the art without departing from the  
6 scope and spirit of the invention. Although the  
7 invention has been described in connection with  
8 specific preferred embodiments, it should be  
9 understood that the invention as claimed should not  
10 be unduly limited to such specific embodiments.  
11 Indeed, various modifications of the described modes  
12 of carrying out the invention which are obvious to  
13 those skilled in the art are intended to be covered  
14 by the present invention.

15

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15            30, 2944.  
16  
17

1     **Claims**

2

3     1.    The use of (i) a naked binding member which  
4    binds to both SCR1 and SCR2 of CD55 or (ii) a  
5    nucleic acid encoding said binding member in the  
6    preparation of a medicament for the neutralisation  
7    of CD55.

8

9     2.    The use of (i) a naked binding member which  
10   binds to both SCR1 and SCR2 of CD55 or (ii) a  
11   nucleic acid encoding said binding member in the  
12   preparation of a medicament for the enhancement of  
13   complement deposition on a tissue.

14

15    3.    The use of (i) a naked binding member which  
16   binds to both SCR1 and SCR2 of CD55 or (ii) a  
17   nucleic acid encoding said binding member in the  
18   preparation of a medicament for treating cancer.

19

20    4.    The use according to claim 3 wherein the cancer  
21   is one or more of colorectal, breast , ovarian,  
22   cervical, gastric, lung, liver, skin and myeloid  
23   (e.g. bone marrow) cancer.

24

25    5.    The use according to any one of the preceding  
26   claims wherein the binding member is an antibody or  
27   a fragment thereof.

28

29    6.    The use according to any one of the preceding  
30   claims wherein the binding member binds to amino  
31   acids 83-93 and SCR2 amino acids 101-112 and amino  
32   acids 145-157 of the sequences shown in Figure 1b.

1     7.     The use according to any one of the preceding  
2     claims wherein the binding member comprises one or  
3     more of the CDRs of the antibody, or a fragment  
4     thereof, produced by the cell line deposited at ATCC  
5     under accession number HB9173.

6

7     8.     The use according to any one of the preceding  
8     claims wherein the binding member is the antibody  
9     791T/36 produced by the hybridoma cell deposited at  
10    ATCC under accession number HB9173.

11

12    9.     The use according to any one of claims 1 to 7  
13    wherein the binding member comprises at least one  
14    human constant region.

15

16    10.    A naked binding member which binds to both SCR1  
17    and SCR2 for use in the treatment of cancer.

18

19    11.    A naked binding member, which binds to both  
20    SCR1 and SCR2 of CD55, and an active agent as a  
21    combined preparation for simultaneous, separate or  
22    sequential use in the treatment of cancer.

23

24    12.    The combined preparation according to claim 11,  
25    wherein said active agent is a Doxorubicin, taxol,  
26    5-Fluorouracil, Irinotecan or Cisplatin.

27

28    13.    The combined preparation according to claim 11  
29    wherein said active agent is an antibody.

30

31    14.    The combined preparation according to claim 13  
32    wherein said active agent is an anti-CD20 antibody;



1 an anti-VEGF antibody; an anti-CD171A antibody; an  
2 anti-CEA anti-idiotypic mAb; an anti-EGFR antibody;  
3 an anti-HMFG anti-idiotypic mAb; an anti-EGFR  
4 antibody, or an anti-HER2 antibody e.g. Herceptin,  
5 Genentech (South San Francisco, CA, USA).

6  
7 15. The naked binding member according to any one  
8 of claims 10 to 11, or the combined preparation  
9 according to any one of claims 12 to 14 wherein the  
10 naked binding member is as defined in any one of  
11 claims 1 to 9.

12  
13 16. A pharmaceutical composition for the treatment  
14 of cancer, wherein the composition comprises a naked  
15 binding member that binds to both SCR1 and SCR2 of  
16 CD55 and a pharmaceutically acceptable excipient,  
17 diluent or carrier.

18  
19 17. The pharmaceutical composition according to  
20 claim 16, wherein the naked binding member is as  
21 defined in any one of claims 1 to 9.

22  
23 18. A method of neutralisation of CD55, comprising  
24 administration of a naked binding member which  
25 specifically binds to SCR1 and SCR2 of CD55.

26  
27 19. A method of enhancing complement deposition  
28 comprising administration of a naked binding member  
29 which specifically binds to SCR1 and SCR2 of CD55.

30  
31 20. A method of treating cancer comprising  
32 administration of a therapeutically effective amount

1 of a naked binding member which specifically binds  
2 to SCR1 and SCR2 of CD55 to a mammal in need  
3 thereof.  
4

5 21. A method according to any one of claims 16 to  
6 18 wherein the naked binding member is as defined in  
7 any one of claims 1 to 9.  
8

9 22. An assay method for identification of an agent  
10 capable of inhibiting CD55 comprising step:  
11

12 a) bringing into contact a candidate agent with at  
13 least a portion of SCR1 and SCR2 of CD55; and  
14

15 b) determining binding of said candidate agent to  
16 both SCR1 and SCR2.  
17

18 23. An assay method for identification of an agent  
19 capable of inhibiting CD55 comprising:  
20

21 (a) bringing into contact a candidate agent with at  
22 least a portion of SCR1 and SCR2 of CD55 in the  
23 presence of a naked binding member which in the  
24 absence of the candidate agent is capable of  
25 binding both SCR1 and SCR2 of CD55; and  
26

27 (b) determining the extent to which the candidate  
28 agent inhibits binding of the naked binding  
29 member to SCR1 and SCR2 of CD55.  
30

1     24. The assay method according to claim 23 wherein  
2     the binding member is as defined in any one of  
3     claims 6 to 9.

4  
5     25. The assay method according to any one of claims  
6     22 to claim 24 further comprising step (c) selecting  
7     a candidate agent which bind both SCR1 and SCR2 of  
8     CD55; and/or step (d) determining the amount of  
9     complement deposition on a cell sample in the  
10    presence and absence of the candidate agent.

11  
12    26. The assay method according to any one of claims  
13    22 to 25 wherein said portion of SCR1 and SCR2 of  
14    CD55 comprises amino acids 83-93, 101-112 and 145-  
15    157 of the sequences shown in Figure 1b.

16  
17    27. Use of an agent identified by the assay method  
18    of any one of claims 22 to 26 in the manufacture of  
19    a medicament for the treatment of cancer.

20

21

**Figure1b. Alignment of CDRs with CD55**

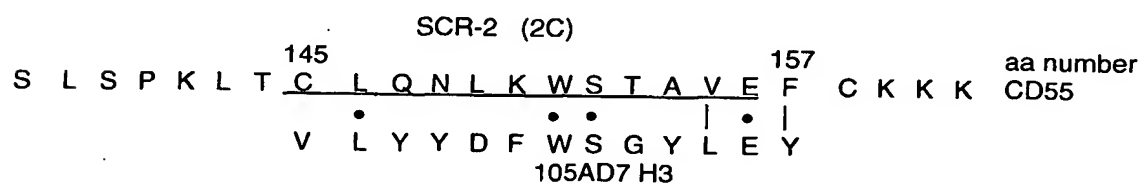


Figure 2

